OUTLINE OF THE PROPOSED WORK FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN PHARMACEUTICAL SCIENCES

A SYNOPSIS

"Formulation and Evaluation of Nano-particulate topical drug delivery system containing Non-steroidal Anti inflammatory drug"

Submitted for the consideration of

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1. Abstract:

Non steroidal anti-inflammatory drug indicated for the relief of inflammation, non steroidal anti inflammatory drug's oral administration contraindicated in patients with peptic ulcer disease, gastro esophageal reflux (GERD) irritable bowel syndrome or other gastrointestinal disorders, controlled release of the drug to the skin could reduce the above mentioned side effects related to oral administered drug formulation while reducing percutaneous absorption. In view of above it was felt worthwhile to produce nanoparticles to control the release of the drug to the skin, nanoparticles will be prepared by nanoprecipitation method, in order to optimize the nanoparticles formulation and factors affecting the physical properties of nanoparticles would be determined and evaluated for various parameters. Drug-excipients compatibility will be performed by FTIR study. Prepared gel formulations would be evaluated for various physical parameters, *in-vitro* drug release kinetics and *in-vivo* acute dermal toxicity study.

2. Introduction:

Nanotechnology is one of the most promising technologies applied in all areas of science. Nanoparticles (NPs) cause attraction of researchers because of their unique properties, owing to their small size (1–100 nm), large surface-to-volume ratio and increased reactivity. Drug delivery from colloidal systems such as nanoparticles dispersed in a gel appears to be unique when compared to the delivery from traditional topical and dermatological formulations. During the last decade, considerable attention has been paid to the development of new controlled delivery systems, in order to supply a long-term drug release and, therefore, increase patient's therapeutic compliance and acceptance. The transdermal drug delivery system can be used to deliver anti-inflammatory drugs across the skin for the treatment of acute and chronic pain and inflammation. Osteoarthritis, rheumatoid arthritis and ankylosing spondyllitis are a group of related, but distinct, disorders of the cartilage of osteoarticular joints. Non-steroidal anti-inflammatory drugs are in use to reduce the pain and inflammation. Their main benefit derives from their anti-inflammatory and analgesic effect, but the use of these agents is not innocuous since their regular use may lead to chronic side-effects such as gastric irritation to severe bleeding and ulceration of gastric due to both inhibition of synthesis of prostaglandins and direct contact of the drug with mucosa produce large side effects. Since long-term NSAID treatment is indicated for such illnesses, the ideal agent should have good efficiency and a low propensity to **c**ause adverse events. Therefore, recent focus of the researchers has been to deliver such potential NSAIDs in a controlled manner by using a dosage form that will minimize its release in stomach and overcomes its chronic adverse effect.

3. Literature Review:

The extensive literature survey revealed the Formulation and Evaluation of Nano-particulate topical drug delivery system containing Non-steroidal Anti inflammatory drug

1. Abitha M H et al (2015)

Authors have revealed that the F3-G1 Flurbiprofen nanoparticle gel can be effectively used for the treatment of chronic conditions of rheumatoid arthritis osteoarthritis.

2. Dhruv K. Purohit et al (2016)

Authors reported the cosmetic benefits including enhancement of chemical stability of actives, film formation, controlled occlusion, skin hydration, skin bioavailability, and physical stability of the lipid nanoparticles. Solid-lipid nanoparticle as topical formulations. List of the cosmetic products currently available in the market, and bioequivalence protocol, excipients, improvement of the benefit/risk ratio of the topical therapy is shown.

3. Abitha M H et al (2015)

Authors reported that Transdermal drug delivery system are a constant source of interest because of the benefits that they afford in overcoming many drawbacks associated with other modes of drug delivery (i.e. oral, intravenous). Becoming more popular due to ease of application and better percutaneous absorption. Gel formulations provide better application property and stability in comparison to cream and ointments. This review is concern with all detail information regarding novel approaches to topical gel formulation, advantages and classification of gel.

4. Kamal Saroha1 et al (2013)

Authors reported the Delivery of drugs through the skin has been an attractive as well as a challenging area for research. Compared to other conventional routes of drug delivery such as oral, injection and inhaler, transdermal delivery has a variety of advantages. Transdermal systems are non-invasive, convenient, and inexpensive and can be self-administered. In these study methods, advantages, gel forming substances, structure of gel, their properties, their absorption mechanism, evaluation and future perspective are discussed to improve the permeability and bioavailability of gels..

5. **A. Dubey et al (2012)**

Authors reported a broad treatment of Nano Structured lipid carriers (NLC) discussing their advantages, benefit/risk ratios and their possible remedies. Different production methods which are suitable for large scale production and applications of Nano Structured lipid carriers (NLC) are described. Appropriate analytical techniques for characterization of Nano Structured lipid carriers (NLC) like Image analysis, differential scanning calorimetry, Zeta Potential, HPLC are highlighted. Modulation of drug release, factors affecting drug release and application areas are also explained with the drugs incorporated in Nano Structured lipid carriers (NLC).If appropriately investigated, Nano Structured lipid carriers (NLC) may open new vistas in therapy of complex diseases.

6. **Yadav et al (2012)**

Authors reported the different preparation techniques available for production of polymeric nanoparticles. It was observed that preparing Polymeric nanoparticals is a state-of-art technology that requires a suitable technique among the various possible methods. The drug-loaded nanospheres or nanocapsules now can be produced by simple, safe, and reproducible techniques available.

7. **Rahul Nair et al (2011)**

Authors reported the latest research developments of the Solid lipid nanoparticles (SLNs) according to the recent relevant literatures were focussed**.** This paper highlights various production techniques for SLNs as well as their advantages and disadvantages. Analytical techniques for characterization of SLNs like electron microscopy, dynamic light scattering, atomic force microscopy, differential scanning calorimetry, X-ray diffraction, nuclear magnetic resonance and electron spin resonance are discussed. Aspects of principles of drug release and different administration routes of SLNs were described.

8. **Aishwarya T. Nair et al (2016)**

Authors reported the various approaches of lipid drug delivery system, different methods of preparation, characterization and applications of Solid lipid Nanoparticles the most prominent and effective Lipid based drug delivery is Solid lipid nanoparticles (SLNs).

9. **Sovan Lal Pal et al (2011)**

Author reported the detail classification, methods of preparation, characterisation, application, advantages of nanoparticles and health perspectives.

10. Loveleen Preet Kaur et al (2013)

Author reported the Topical application of drugs offers potential advantages of delivering the drug directly to the site of action and acting for an extended period of time. Topical gels are intended for skin application or to certain mucosal surfaces for local action or percutaneous penetration of medicament for their emollient or protective action. Gels are evaluated by following parameters such as pH, homogeneity, grittiness drug content, viscosity, spreadability, extrudability, skin irritation studies, in-vitro release, in Stability

11. VJ Mohanraj et al (2006)

Author reported the various aspects of nanoparticle formulation, characterization, effect of their characteristics and their applications in delivery of drug molecules and therapeutic genes.

12. P.K. Lakshmi et al (2011)

Author reported the therapeutic effectiveness of ibuprofen by increasing its transdermal permeation, via solid dispersion incorporated in gel. Solid dispersion ibuprofen formulation incorporated in gel produced better results than other formulations prepared with permeation enhancers.

13. Doaa A. Helal et al (2012)

Author reported the different formulation of topical gel containing fluconazole for treatment of fungal infection of skin. Formulation were prepared and characterized physically in term of color, syneresis, spreadability, pH, drug content and rheological properties. Drug-excipients compatibility studies were confirmed by carrying out DSC and FT-IR. In-vitro drug release in phosphate buffer pH 5.5 and permeation study through cellulose membrane, using a modified Franz diffusion cell, were performed. In this study F3 formulation shows the highest antifungal activity.

4. Aim and Objective:

Aim:

Formulation and Evaluation of Nano-particulate topical drug delivery system containing Non-steroidal Anti-inflammatory drug.

Objectives:

The objectives of present study are

1. To develop and establish safe, ecofriendly, cheaper method for the Nanoparticles.
2. To reduce the amount of dose.
3. To prolong the release over a predetermined time period.
4. To decreases the side effects and effectively management of topical drug delivery system
5. To study stability of Nanoparticulate topical gel in different pH range.
6. To improve the bioavailability of drugs.

5. Methodology to be adopted:

1. Literature survey.

2. Selection and Collection of Drugs & excipients

   2.1. Drug: Anti-inflammatory drugs: (Celecoxib, Naproxen)

   2.2. Selection of Excipients: Viscosity modifiers, Gelling agent, Penetration enhancers, Solvents, Preservatives etc.

3. Experimental Work:

3.1. Preformulation studies:

- 3.1.1 Solubility analysis
- 3.1.2 Melting point
- 3.1.3 Partition coefficient
- 3.1.5 UV spectral analysis
- 3.1.6 Organoleptic properties
- 3.1.7 X-Ray Diffraction (XRD) analysis

3.2. Drug excipients compatibility study:

- 3.2.1. FT-IR spectral analysis
- 3.2.2. Differential scanning calorimetry (DSC)

3.3. Preparation and Evaluation of Nanoparticulate topical gel:

Step I: Preparation and Evaluation of Nanoparticles:

Preparation of Nanoparticles by Nanoprecipitation method:

Nanoparticles were prepared by nanoprecipitation method. Drug and polymer were dissolved in ethanol: Dichloromethane mixture by using mechanical stirrer. This organic phase added drop by drop (2 ml/min) in external aqueous phase containing surfactant tween 60 in a fixed concentration. During this mixing, the aqueous phase was homogenized using homogenizer at 10,000 rpm for 30 minute followed by magnetic stirring for 3hrs and kept overnight. The

formed nanoparticles suspension were filtered through whatman filter paper and washed nanoparticles were dried.

Evaluation of Nanoparticles:

1 Particle size
2 Shape and surface morphology
3 Percentage yield
4 Entrapment efficiency
5 *In-vitro* Drug Release Study:

In-vitro release was evaluated using a dialysis bag technique. The *in- vitro* release of nanoparticles was carried out in stirred dissolution cells by suspending Nanoparticulate suspension into a beaker containing 100ml of release media: phosphate buffer saline pH 7.4. Then, the beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 0.5°C throughout the study. Samples (5 ml) were withdrawn at definite time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11and 12 hrs) and replaced With equal amounts of fresh buffer. The samples were analyzed for drug concentration by UV-Vis spectrophotometer.

Step II: Preparation & Evaluation Nanoparticulate topical gel:

Preparation of Nanoparticles loaded carbopol gels:

Gel forming polymer (Carbopol) was soaked in water for 24 hours and then dispersed by agitation to get a smooth dispersion. The dispersion was allowed to stand for 15 min to expel entrapped air. Simultaneously nanoparticles, propylene glycol, permeation enhancer was added to water and undergoes gentle stirring. This was added to carbopol mixture by stirring, triethanolamine is added to form gel.

Evaluation of Nanoparticulate topical gel:

1. Physical Examination
2. Drug content determination
3. pH determination
4. Spreadability
5. Viscosity measurements and rheological behavior

6. *In-vivo* Studies.
7. *In Vitro* drug release kinetics:

Preparation of cellophane membrane/ Mice Skin for the diffusion studies:

The cellophane membrane /Mice skin was washed in the running water. It was then soaked in distilled water for24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies.

Procedure:

The *in vitro* diffusion studies of prepared gel were carried out in hollow tube diffusion cell using prehydrated cellophane membrane / Mice skin. 100 ml of phosphate buffer of pH 7.4 was used as receptor compartment, and then 1g of gel containing nanoparticles was spread uniformly on the membrane/ skin facing the donor compartment. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±100C. The buffer solution was kept on the receptor side. At pre determined time intervals, pipette out 5ml of solution from the receptor compartment and immediately replaced with the fresh 5ml phosphate buffer. The drug concentration on the receptor fluid was determined spectrophotometrically.

8. Skin Irritation study on rabbits:

To test the irritancy potential of experimental Topical gel rabbits were used; the selected gels were applied to the shaved skin on the rabbits back. The selection of rabbits for this study is described as follows.

Test animal	– Healthy Rabbits
Strain	– New Zealand white rabbits
Age	– 22 weeks
Sex	– Male
Weight	– 2-2.5 kg
No of animals per group	– 3

Selection and Randomization:

Animals were randomly placed in cages upon receipt and then randomized according to the body weights, Animals considered unsuitable because of outlaying body weights were excluded from the study. The animal backs were carefully shaved using sterilized shaving blade to remove the fur. The selected animals were grouped into 4 groups. and were marked with identification codes using diluted picric acid solution (1.2%w/v). Circular areas of 2.54 cm (1 inch diameter) were marked on the back of each animal using marker ink one spot on right side and one spot on left side of vertebral column.

The spot on right side was abraded using shaving blade and the spot on left side was kept intact 1gm quantity of the selected gels was applied on marked spots using absorbent cotton wool. The toxic manifestations if any on the skin were then assessed by observing the skin at pre-selected time intervals after treatment e.g. 1 hrs, 4 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hours,

9. Comparison with marketed formulation
10. Stability studies.

    Optimized formulation was selected and kept for stability studies. Formulations were packed in a glass bottles and studies were carried out for 30 days by keeping at 35^0C, 40^0C.

6. Importance of study /society application:

Topical gels of NSAIDs are very useful as palliative products for treating pain &inflammation associated with arthritis, alkalosis etc. Their greater advantage seems to be the obviation of gastrointestinal distress caused when the same drugs are given orally.

NSAID considered superior to its selective action on COX-2 enzyme within the inflamed tissue only. Hence it was decided to formulate a simple yet effective gel of it, recently one or two similar products have been introduced in market however not much is known about their formulation aspects. Various synthetic gelling agents are available with lot of literature suggesting their safety & suitability for topical use.

The idea was to formulate nanoparticles topical gel with each of them & compare the gels for various physical, chemical & biological properties. Also the effect of penetration enhancers was compared for the selected formulations.

7. Proposed work plan:

Sr. no	Activity	Months
1.	Literature survey	03
2.	Selection and Collection of Drug and Excipients	03
3.	Preformulation Studies	03
4.	Formulation of Nanoparticulate topical gel	06
5	Evaluation of Nanoparticulate topical gel 5.1. Physicochemical Evaluation 5.2. Biological Evaluation 5.2.1. *In Vitro* drug release kinetics. 5.2.2. *In-vivo* Studies 5.2.3. Skin Irritation study	09
6.	Statistical Analysis, Data Interpretation and Conclusions	03
7.	Stability studies of Nanoparticulate topical gel	03
8.	Communication with Research Journals	03
9.	Submission of report	03
	Total	**36**

8. References:

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